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HISTOLOGICAL AND PHYSIOLOGICAL ASPECTS OF THE MEDULLA EXTERNA X ORGAN, A NEUROSECRETORY CELL GROUP IN THE EYESTALK OF *PALAEMON SERRATUS* PENNANT (CRUSTACEA, DECAPODA, NATANTIA)

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Abstract

The medulla externa X organ (MEX) is composed of two cell types containing two different categories of neurosecretory elementary granules also found in the sinus gland. Changes in the volume and activity of the organ were observed in relation to the intermolt cycle and season. After extirpation of the medulla externa X organ, the volumes of both the sinus gland and the organ of Bellonci increased in post- and intermolt stages. In the sinus gland, lysis and clumping were observed in axon endings containing one (large) granule type. Also as a consequence of the extirpation, large droplets were secreted in vacuoles of the organ of Bellonci. Neurosecretory cells in the medulla terminalis ganglionaris X organs (MTGX) showed increased activity and hypertrophy. The medulla externa X organ also had an impact on the distal retinal and chromatophoric red pigments.

Introduction

The eyestalks in decapod crustaceans are complex appendages considered to be the main source of hormones acting in several physiological and developmental processes.

The anatomy and cytology of the nervous and neurosecretory structures of the optic ganglia, well known since Hanström's work (1928, 1947), have been described in numerous light-microscopic investigations. However, few studies deal with changes in the neurosecretory pathways of decapods after surgical interruptions. Several authors have been successful in removing the sinus gland in decapods (Brown, 1942; Scudamore, 1942; Panouse, 1944; Kleinholz, 1947; Stephens, 1951). But only Passano (1951a) and Bliss and Welsh (1952) observed changes so produced in eyestalk structures. Extirpation of the neurosecretory cell centers, carried out by Bliss and Welsh (1952) and by Passano (1953), gave a first idea of the histological changes induced. Since then, no selective surgical experiments on the neurosecretory cell groups in the decapod eyestalk have been reported, although the removal of the organ of Bellonci and the medulla externa X organ (MEX) in the Natantia *Palaemon serratus* is technically possible (Pasteur, 1958).

Our objective was to determine whether the chromatophore index and the blood

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Abbreviations: CHH: Crustacean hyperglycemic hormone; DRPH: Distal retinal pigment hormone; MEX: Medulla externa X organ; MTGX 1 and 2: Medulla terminalis X organs 1 and 2; P.A.F.: Paraldehyde fuchsin; RPCH: Red pigment concentrating hormone.

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sugar content of prawns change after extirpation and injection of the MEX organ. Special attention was paid to the neurosecretory characteristics of the MEX cells, and to exogenous and endogenous factors that eventually modulate these cells. Effects on neurosecretory eyestalk structures remaining after removal of the MEX organ were also studied.

Materials and Methods

Collection and maintenance of specimens

Palaemon serratus Pennant adults were collected during February, April, July, and November of 1977, in the Bay of Concarneau (France). They were maintained in translucent containers with recycled aerated sea water (pH 8.0; 30‰ salinity). Room temperature varied between 17°C in winter and 22°C in summer. The natural photoperiod was season-bound and ranged between 14 h darkness:10 h light in February, 9½ h darkness:14½ h light in April, 8 h darkness:16 h light in July, and 12 h darkness:12 h light in November. The light intensity above the aquaria varied between 800 and 2800 lux in February, 800 and 4000 lux in April, 8000 and 16000 lux in July, and 600 and 3000 lux in November. The animals were fed granulated food, fresh mussels, and fish. For each experiment, prawns were selected according to molting stage, sex, and size.

Procedure for light microscopy

Eyestalks were removed between 3 and 4 p.m. and fixed according to Halmi (Gabe, 1968) for 24 h. After dehydration in 95% ethanol and n-butanol (3 changes for 3 h in each medium), they were embedded in Histomed (58°C melting point). Sections 7 μm thick (transversal and longitudinal) were stained with the azan novum method of Geidies (Gabe, 1968). Masson's and Gabe's trichrome staining methods were also used. Neurosecretory cells were stained with paraldehyde fuchsin, according to Gabe (1953).

Morphometric measurements were carried out with a "Manuell Optisches Bild Analyse System (MOP) Kontron" for determining areas and diameters. Volumes of structures were calculated using the following formula: $(t \times d \times k)/m$, where t = total area, d = thickness of the section, k = order of selection of the sections, and m = magnification, as previously described (Van Herp *et al.*, 1977).

Numerical data were worked out statistically by Student's *t* test.

Procedure for electron microscopy

For transmission electron microscopy, extirpated organs were immediately fixed in cold 2.5% glutaraldehyde buffered with 0.1 *M* sodium cacodylate, pH 7.3; and postfixed in 1% osmium tetroxide dissolved in the same buffer. After dehydration in an ascending series of ethanol concentrations, the organs were embedded in a mixture based on Epon 812 (sol. A: Epon 812 (62 cc) + dodecenyl succinic anhydride (100 cc); sol. B: Epon 812 (100 cc) + methyl nadic anhydride (89 cc); mixture: 5 cc sol. A + 5 cc sol. B + 0.15 cc DMP_{30}). Silver to gray ultrathin sections were cut with a Reichert O.M.U. 2 Ultramicrotome. After poststaining with uranyl acetate (Watson, 1968) and lead citrate (Reynolds, 1963), the sections were examined with a Philips 300 electron microscope at 60 kV.

For scanning electron microscopy, eyestalks were first washed in 0.1% ammonia in distilled water before fixation in absolute ethanol-chloroform (2:1) for 1 week.

They were rinsed in absolute ethanol and in n-butanol (3 changes in each medium). After gold-palladium coating, samples were examined with a Cambridge S 4 electron microscope. Best resolution was obtained at 20 kV with a current of 150 μA.

Technique of MEX extirpation in living prawns

The prawn was placed on its ventral side into a notch in a cork plate and strongly attached there with a flexible sheet of plastic. Using a dissection microscope (magnification: ×25), the left eye of the prawn was pulled back from the rostrum. Four perforations were made with a sharp stainless steel needle in the rostrodorsal area of the eyestalk below the ommatidia. A cuticular piece (0.25 mm^2) was cut away. The underlying epidermis was then removed with an iridectomy knife. Because of its thickness, the connective tissue sheath covering the MEX organ was carefully extirpated with sharp tweezers, revealing the bluish-white MEX organ attached to the medulla externa. Under slight pressure it was lifted and picked up with tweezers. A superficial cauterization was necessary to limit bleeding and infection.

The whole extirpation required about 8 min. Twenty-four h later, the right eyestalk was cut off and the stub cauterized.

Histological preparations showed that the optic ganglia were not impaired. Healing tissue was found in the operated area. Sham operations showed that ommatidia were not directly damaged by the operation. Normal retinal pigment migrations were observed the first day after the extirpation in all operated animals. They persisted after sham operations, even without cauterization.

All extirpations were carried out during stage C, according to the definition of molt stages by Drach and Tchernigovtzeff (1967). The histological investigations were carried out after the next molt.

Survival was good: mortality did not exceed 5% during the first week, and reached 30% 1 month after the following molt.

Physiological assays

Chromatophore index estimation: After extirpation, the red pigment in the chromatophores was examined daily. The measurements were recorded in Panouse's scale (Panouse, 1946), using six stages to describe pigment dispersion (0: full pigment concentration, rounded chromatophore; 1: irregular shaped chromatophore; 2: stellate chromatophore with some large branches; 3: sea-urchin-like aspect with bifurcated chromorhizae; 4: highly bifurcated and fine chromorhizae, chromatophores still distinct; 5: full dispersion, adjacent chromatophores with intermingled chromorhizae). Measurements were always made in the dorsal area of the first abdominal segment at 10 and 12 a.m., on pools of 10 intact and 10 operated animals. From these values, the mean daily degree of dispersion was calculated, in order to relate pigment movement to time.

The circadian rhythm was studied on groups of 15 intact and 15 operated animals. For this purpose, the pigment index was evaluated every 2 h (daytime) or 3 h (nighttime) on the first and the eighth day after the operation.

Crude MEX extracts were prepared in sea water, centrifuged at 3000 *g* for 20 min and injected into eyestalkless intermolt prawns (0.2 ml/animal). Sixty min were allowed for measurements. Chromatophores were also examined after injection of either boiled or lyophilized extracts.

Glycemia measurements: Blood samples were collected by cutting off the telson.

The samples were pooled, frozen for 48 h, thawed, and centrifuged. Glucose was measured in the serum using the "GOD perid" test kit (Boehringer, Mannheim) and expressed as $\mu g/ml$.

In extirpation experiments, blood samples were collected at 12 a.m. from operated animals in the premolt stages (D'_1–D'''_1) and again 12 and 48 h after operation. For injection purposes, the MEX material was homogenized in distilled water at 4°C, extracted for 2 h, and lyophilized. The equivalent of one organ was suspended in 50 μl (⅓ distilled water: ⅔ sea water) and injected into the abdomen of an eyestalkless intermolt prawn. Blood samples were taken 2 h later.

Results

General description of the MEX organ

Morphological observations: The MEX organ is a well defined group of cells located dorsolaterally on the rostral side of the eyestalk and at the surface of the medulla externa, opposite the sinus gland and facing the upper part of the organ of Bellonci, which lies on the ventral side (Figs. 1, 2, 3, 4).

At the base of the organ there seems to be a nervous connection with the ventral region of the sensory pores, near the organ of Bellonci (Fig. 3). In addition, axons from the distal MEX cells joining the lamina ganglionaris were identified microscopically. The relationship with the sinus gland awaits confirmation from iontophoresis experiments: axons, tangential to the medulla externa, pass to the distal

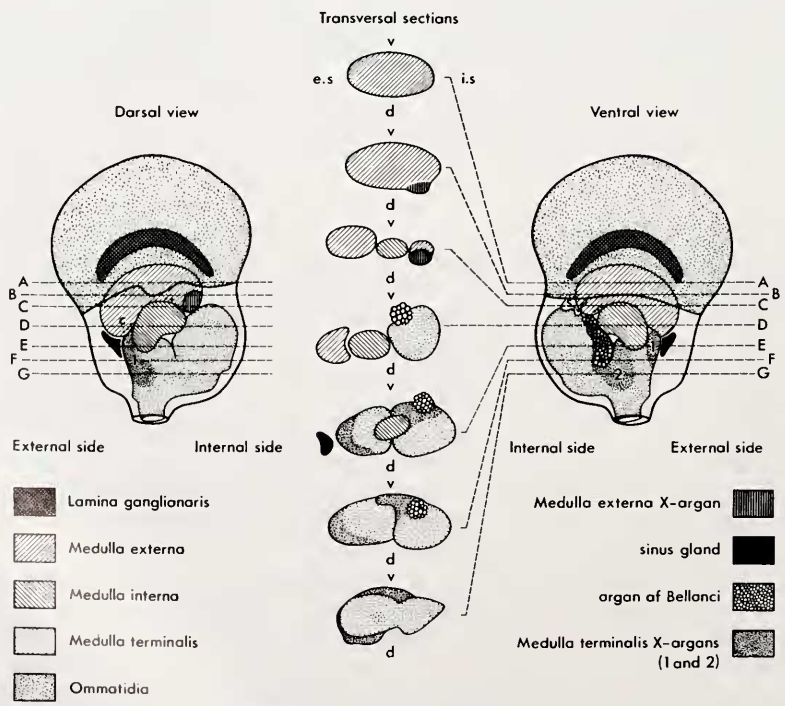


FIGURE 1. General organization of a left eyestalk of the prawn *Palaemon serratus*: dorsal and ventral views, transverse sections. (d: dorsal side; v: ventral side; e.s.: external side; i.s.: internal side; c (left): scattered neurosecretory cells; s (right): sensory pores; A, B, C, D, E, F, G: transverse sections).

part of the gland. A previous publication (Van Herp *et al.*, 1977) described the light-microscopic characteristics of the cell types in the MEX organ. Five cell types could be distinguished by appropriate staining and measuring the cell bodies and nuclei. Some of these cells are shown in Figure 6.

Electron micrographs show two types of neurosecretory cells in the MEX organ. They differ in elementary granules and the endoplasmic reticulum.

Type 1 cells have electron-dense elementary granules with a mean diameter of 130 nm. They are rather scarce, in spite of the abundant Golgi apparatus and ribosomes. The rough endoplasmic reticulum has well developed lamellae. The smooth endoplasmic reticulum consists of heavily packed tubules. Mitochondria are few. The nuclei are very dense and irregular (Figs. 7, 9).

Type 2 cells contain granules of about 96 nm diameter and much lower electron density. Rough endoplasmic reticulum is abundant throughout the cytoplasm. In contrast to the first cell type, mitochondria are very numerous. Active Golgi complexes showing condensing vacuoles and multivesicular bodies are apparent in some cytoplasmic areas. The round nuclei show areas with very dense chromatin (Figs. 8, 10).

The two cell types appear to have different locations in the organ: the first cell type is more common at the edge of the organ; the second is more abundant in contact with the medulla.

Morphometric observations: To relate activity of the MEX organ to molting cycle, season, sex, and size of animals, the volume of the organ was measured and the number of cells larger than 20 μm was determined (Table I). The organ itself does not seem to change much in volume during different stages of the molting cycle, but a slight yet significant change was observed in the number of active neurosecretory cells: They are more numerous in postmolt and intermolt than in premolt. No significant seasonal influences on the MEX organ could be detected, except for a slight increase in volume during summer. In small prawns the large neurosecretory cells seemed slightly less numerous. No other significant differences due to sex or size were noticed.

Histophysiological impact of MEX organ extirpation

Morphological observations in the eyestalk: The new cuticle showed no trace of the wound after the postmolt, nor was there a gap in the epidermis inside the eyestalk. The optic ganglia appeared normal. Connective tissue and large blood lacunes filled the area of extirpation.

After the extirpation, the sinus gland appeared large and clear, in contrast with its milky appearance in controls. Microscopic examination revealed no important changes, except that orange azan-stained clumps of droplets in the lacunar spaces of the inner blood sinus appeared more numerous, chiefly in stage B.

The possible impact of the operation on the sinus gland was also studied with the electron microscope. A previous publication (Strolenberg *et al.*, 1977) described five granule types with mean diameters of 70, 79, 98, 125, and 150 nm. In the present study, the frequency of granule types smaller than 100 nm in stage C control animals was 57%. Stage C experimental animals had all five types of granules, and about the same frequency distribution (60% granules $<$ 100 nm in diameter). However, granules of 125-nm mean diameter had undergone lysis and clumping (Fig. 11). The outer wall or basement membrane of the gland also seemed thicker after the operation.

As shown in Table II, the sinus gland was twice as large after MEX organ

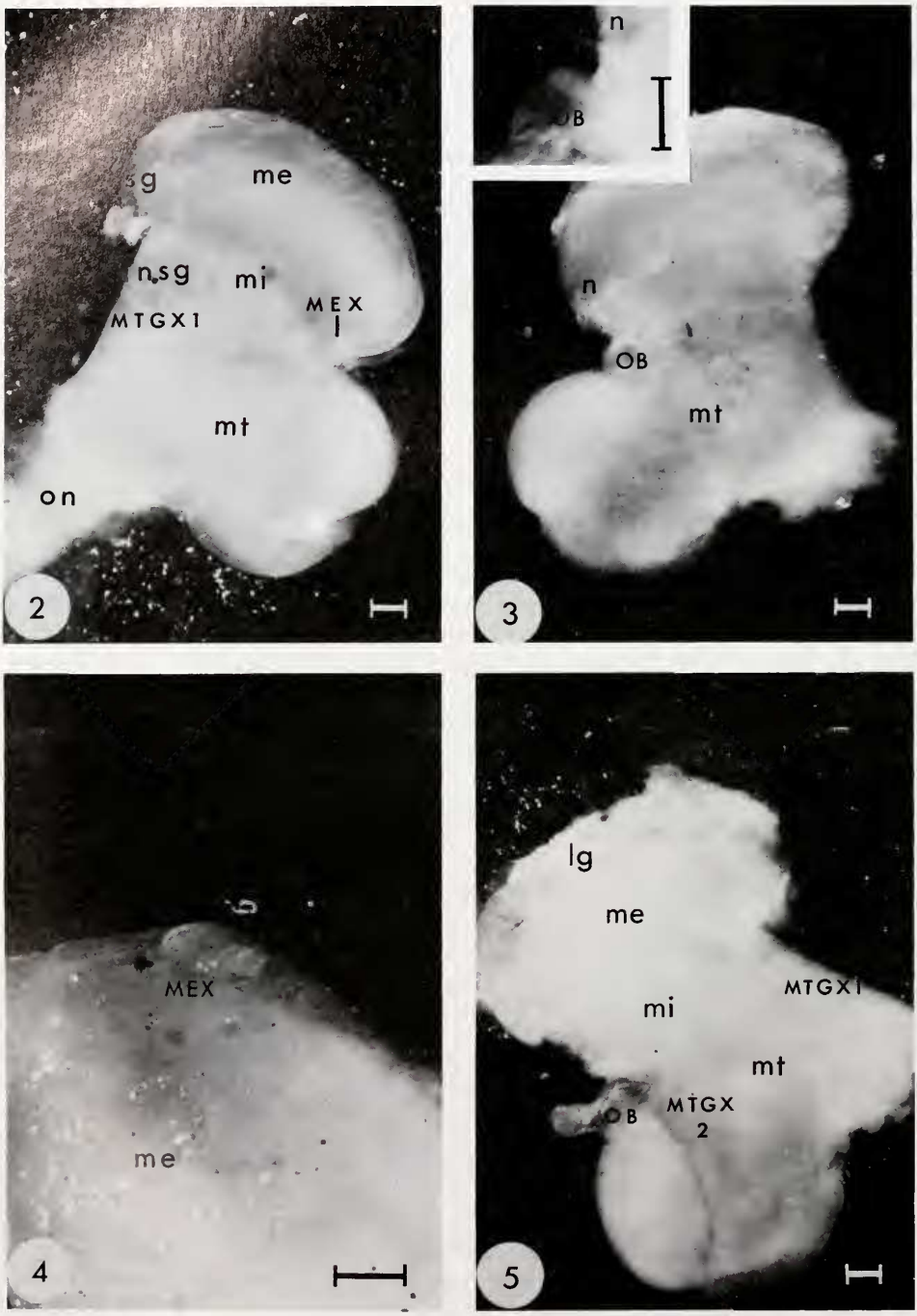


FIGURE 2. Dorsal view of a left eyestalk, showing the medullae (me: medulla externa, mi: medulla interna, mt: medulla terminalis), the optical nerve (on), the sinus gland (sg) with its two clearly distinct parts and its nerve (n.sg), and the locations of the MEX and MTGX 1 organs. Scale bar equals 200 μm.

FIGURE 3. Ventral view of a left eyestalk showing the nerve (n) of the medulla externa and the organ of Bellonci (OB), extruding from the medulla terminalis (mt). The inset shows that the nerve does not join the organ of Bellonci. Scale bar equals 200 μm.

extirpation in post- and intermolt animals and comparable in volume to the sinus gland in the premolt stages. The operation had no visible effect on the last stages.

The neurosecretory cells of *Palaemon serratus* are found in the medullae externa and terminalis. In the medulla terminalis they form two groups: the MTGX 1 and MTGX 2 organs. The former is located on the outer side of the ganglia below the sinus gland and the latter lies on the ventral side below the organ of Bellonci (Figs. 1, 2, 5). These groups are characterized by four cell types (Van Herp *et al.*, 1977). Our morphometric studies were concerned with these two organs. A few cells scattered on the ventral side of the medulla externa were not included in the measurements.

Activity of the neurosecretory cells in the MTGX 1 and MTGX 2 organs was measured by counting cells with a diameter larger than 20 μm in control and experimental animals.

Active cells were more numerous during the postmolt and intermolt stages of control and operated animals (Table II). Moreover, MEX extirpation induced cell hypertrophy during these stages: In extirpated animals large cells were significantly more numerous. This hypertrophy was particularly striking in the area of the giant neuron, located in the apical region of the MTGX 1 organ.

Under light microscopy, the lobular structure of the organ of Bellonci in extirpated animals resembles that described for controls. It consists of packs of onion bodies in the distal part and degenerating onion bodies in the proximal part, near the MTGX 2 organ. Onion bodies are typical of the organ of Bellonci: They appear as coiled outer ciliary segments of sensory cells. In these bodies, degenerative processes induce the formation of a central vacuole, filled with an undefined material. In controls, various stages of lysis of onion bodies were observed in these central vacuoles, as shown by droplets of secretion that stained pale blue with azan or light purple with paraldehyde fuchsin (P.A.F.) (Fig. 14). In contrast, in the extirpated animals, the proximal part contained large vacuoles filled with orange azan-stained material, like the aforementioned droplets in the sinus gland. Droplets were also observed outside the organ of Bellonci next to the MTGX 2 organ. These droplets correlated with the molting cycle: They were large during stage B and appeared more granulated in stage C. Only a few were observed in stage D (Figs. 15, 16).

A morphometric estimate of the volume of the entire organ of Bellonci suggests an increase after the first molt in operated animals. This phenomenon became particularly evident during the post- and intermolt stages (Table II).

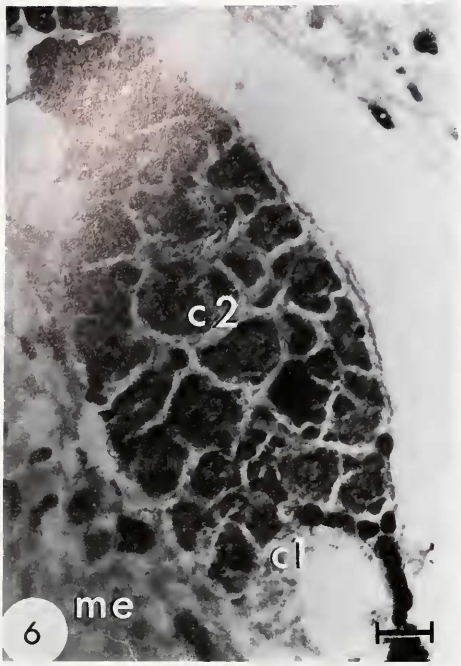
After the molt following extirpation, there were striking changes in the main and larval sensory pores, located on the rostroventral side of the eyestalk under the ommatidia. In some cases it became impossible with scanning electron microscopy to distinguish the minute double micropores of the main sensory pore and the single micropores of the larval sensory pore. Occasionally, only the large rectangular site of the main sensory pore and the circular impression of the larval sensory pore could be detected (Figs. 12, 13).

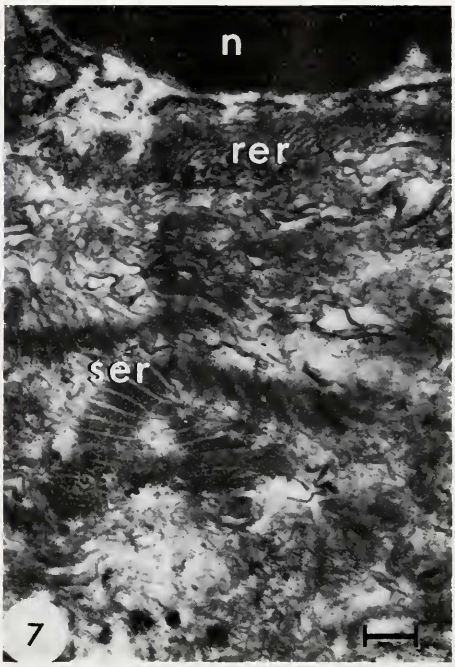
Physiological observations

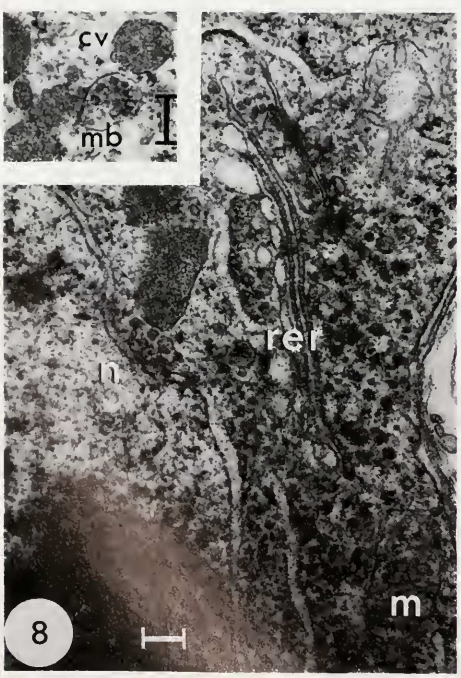
Retinal pigment migration: The day after MEX organ extirpation and before sectioning of the second eyestalk, the dark coloration of the operated eye was

FIGURE 4. View of the MEX organ at the edge of the medulla externa (me). Scale bar equals 200 μm.

FIGURE 5. Ventral view of a left eyestalk, showing the lamina ganglionaris (lg) and the medullae (me, mi, mt), the prominent organ of Bellonci (OB), and the locations of the MTGX organs 1 and 2. The MTGX 1 lies on the external side, under the sinus gland. The MTGX 2 is on the ventral side in contact with the organ of Bellonci. Scale bar equals 200 μm.







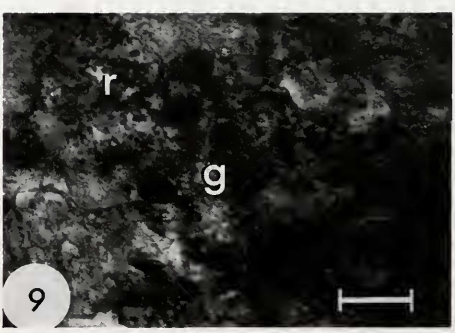


FIGURE 6. Longitudinal section of the MEX organ in light microscopy, showing the neurosecretory cell group (lying against the medulla externa (me)) with its two types of cells: C 1, with dark and irregular nuclei; C 2, with clear and round nuclei. Scale bar equals 25 μm.

FIGURE 7. Ultra-thin section of type 1 neurosecretory cell, with nucleus (n), rough (rer) and smooth (ser) endoplasmic reticulum. Scale bar equals 0.25 μm.

TABLE I

Variations in the MEX organ according to intermolt cycle, season, sex, and size. Level of significance: p > 0.1 = not significant (n.s.); 0.05 < p < 0.1 = barely significant (± s.); 0.01 < p < 0.02 = significant (s.); 0.001 < p < 0.01 = highly significant (h.s.).

Parameters X = ±SEM	Volume of MEX (mm^3) ± SD		Number of cells >20 μm in MEX ± SD	
Intermolt cycle				
Postmolt (A–B)	0.29 ± 0.04	N = 5	46 ± 7	N = 5
Intermolt (C)	0.28 ± 0.03	N = 2	45 ± 11	N = 2
Premolt ($D°–D'''_1$)	0.30 ± 0.04	N = 10	33 ± 3	N = 10
Student's *t* test between different molting stages	all correlations n.s.		$A–B/D°–D'''_1$ ± s. $A–B–C/D°–D'''_1$ ± s. other correlations n.s.	
Seasons				
Feb. 13, 1977	0.22 ± 0.01	N = 2	35 ± 6	N = 2
March 26, 1977	0.27 ± 0.03	N = 11	37 ± 4	N = 11
July 20, 1977	0.37 ± 0.04	N = 3	46 ± 9	N = 3
Nov. 11, 1977	0.43	N = 1	42	N = 1
Student's *t* test between seasons	February/July ± s. other correlations n.s.		all correlations n.s.	
Sex				
Males	0.29 ± 0.04	N = 8	43 ± 4	N = 8
Females	0.31 ± 0.04	N = 8	36 ± 5	N = 8
Student's *t* test between sexes	n.s.		n.s.	
Size				
⩽50 mm	0.28 ± 0.04	N = 6	31 ± 2	N = 6
50<, <60 mm	0.27 ± 0.03	N = 6	44 ± 5	N = 6
⩾60 mm	0.33 ± 0.07	N = 5	41 ± 5	N = 5
Student's *t* test between different sizes	all correlations n.s.		T ⩽ 50 mm/50 < T < 60 mm ± s. other correlations n.s.	

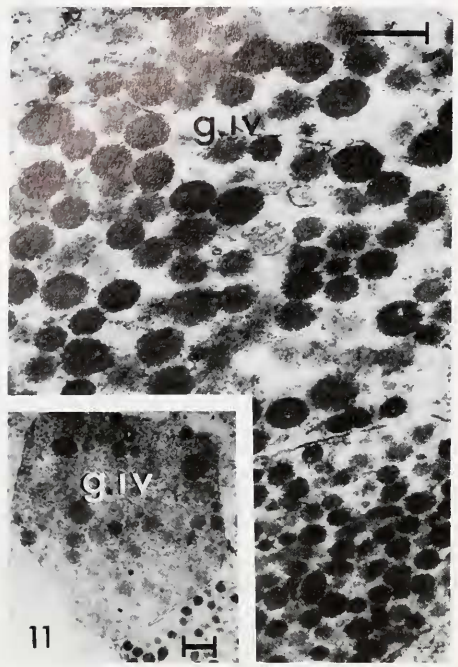
striking when compared to the clear intact eye. In fact, the distal retinal pigment, which usually migrates towards the periphery before darkness, continued to display its night adaptation and never moved during the period of observation. This provided a criterion for a successful operation. This effect was not noticeable after an incomplete MEX organ extirpation, nor after a sham operation or sinus gland extirpation. Its absence after sham operations shows that the effect was not caused by cauterization or bleeding.

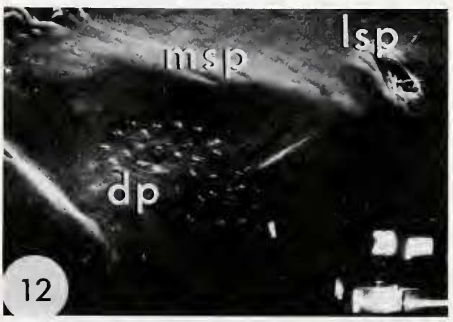
Chromatophore red pigment migration: Three types of chromatophores in the

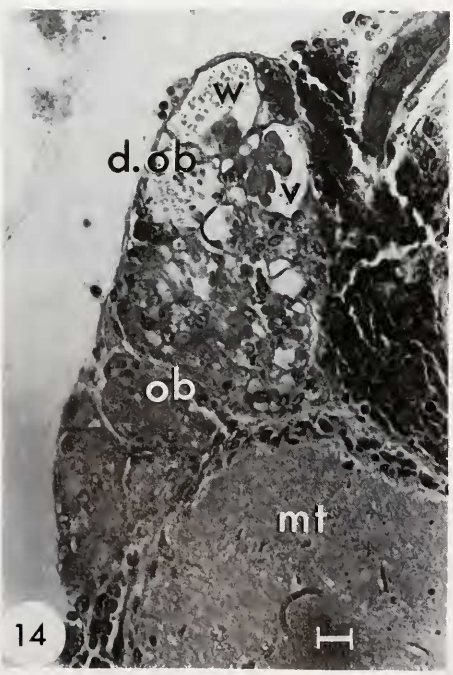
FIGURE 8. Ultra-thin section of a type 2 neurosecretory cell, with nucleus (n), rough endoplasmic reticulum (rer) and mitochondria (m). In the inset, an example of multivesicular body (mb) and condensing vacuole (cv). Scale bars equal 0.25 μm.

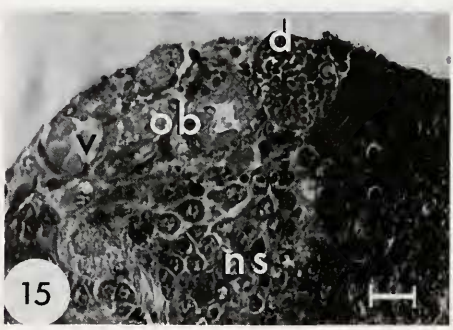
FIGURE 9. Granules (g) of 130-nm diameter (d), from cell type 1. Note their high electron density and the abundance of ribosomes (r). Scale bar equals 0.25 μm.

FIGURE 10. Granules (g) of 96-nm diameter, from cell type 2. In this cell type, Golgi cisternae (GC) are well developed. Scale bar equals 0.25 μm.









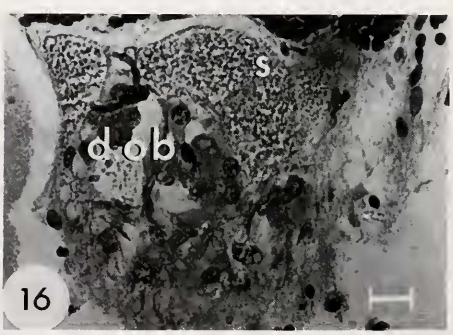


FIGURE 11. Ultra-thin section of the sinus gland, showing an axon terminal with granules of 125-nm diameter (g IV) in a control animal. Lysis affects this type of granule after MEX extirpation, as shown in the inset. Scale bars equal 0.25 μm.

FIGURE 12. SEM view of the sensory pores in a control animal (stage C) showing the main sensory pore (msp) with its double micropores (dp) and the larval sensory pore (lsp).

TABLE II

Impact of MEX organ extirpation on the eyestalk structures during the intermolt cycle following the operation. Level of significance: See Table I.

Parameters	A + B + C	D°–D‴	A–D‴	Student's *t* test intermolt cycle
	Volume of the sinus gland (mm^3) ± SD			
Controls	0.27 ± 0.03 N = 7	0.55 ± 0.11 N = 5	—	s.
Operated	0.55 ± 0.10 N = 6	0.62 ± 0.22 N = 4	0.58 ± 0.10 N = 10	n.s.
Student's *t* test operation	s.	n.s.	—	
	Number of cells > 20 μm in MTGX organs 1 and 2 (±SD)			
Controls	33 ± 2 N = 3	8 ± 1 N = 3	—	h.s.
Operated	54 ± 4 N = 4	15 ± 7 N = 2	—	h.s.
Student's *t* test operation	s.	n.s.	—	
	Volume of the organ of Bellonci (mm^3) ± SD			
Controls	1.21 ± 0.01 N = 2	1.38 ± 0.19 N = 7	1.23 ± 0.10 N = 9	n.s.
Operated	2.01 ± 0.28 N = 6	1.47 ± 0.12 N = 2	1.88 ± 0.2 N = 8	n.s.
Student's *t* test operation	± s.	n.s.	h.s.	

hypodermis of *Palaemon serratus* contain red pigment. One type forms stripes in a specific pattern on the body. The second is composed of smaller units scattered in the intervals between stripes. In both instances a yellow pigment is also present. The third type is a large chromatophore which also contains a white pigment. Its location does not vary and its behavior seems more complex. This type of chromatophore is not included in the present study.

FIGURE 13. A comparable view of the main sensory pore (msp) in a MEX extirpated animal in postmolt following the operation. The aperture of micropores is no longer visible.

FIGURE 14. Light-microscopy of a transverse section of the organ of Bellonci in a control animal, at the level of medulla terminalis (mt), with packs of onion bodies (ob), degenerating onion bodies (d.ob) and their waste (w) filling vacuoles (v). Scale bar equals 25 μm.

FIGURE 15. Section in light microscopy of the organ of Bellonci in a MEX extirpated prawn (stage B), showing onion bodies (ob) and large droplets (d) filling spaces in the organ. Note also the presence of neurosecretory cells (ns) of MTGX 2 in close contact with the organ of Bellonci. Scale bar equals 25 μm.

FIGURE 16. Section in light microscopy of the organ of Bellonci in a MEX extirpated prawn (stage C), showing the degenerating onion bodies (d.ob). A thin granular secretion (s) here takes the place of droplets. Scale bar equals 25 μm.

After extirpation of the MEX organ and sectioning of the remaining eyestalk, the red pigment in the chromatophores began to expand. Pigment dispersion increased for several days (Fig. 17). The speed of the reaction was linked to several factors, such as temperature, photoperiodicity, and molting stage. The chromatophores also displayed diurnal pulsations.

As a rule, operated animals became reddish brown after the molt following the operation, but a few animals remained pale. The eyestalks of these prawns invariably displayed a few non-extirpated MEX cells or some large neurosecretory cells on the ventral side of the medulla externa. In general, their MTGX organs appeared more developed.

Compared with MEX organ extirpation, sinusglandectomy immediately induced a strong dispersion of the red pigment which persisted for only a short while. Chromatophores contracted progressively from the second or third day after the operation.

As shown in Figure 18, the circadian rhythm in control animals was biphasic: on a white or sandy background, the chromatophores, especially on stripes, were contracted during the night and more or less dispersed during the day. In operated animals this rhythm was lost, the loss being related to an increased dispersion; and on a white background, the rhythm tended to be reversed. Pigment was concentrated early in the morning and dispersed before sunset. Operated animals became more colored during the night than during the day. These responses could be detected when the animals turned darker, a few days after the operation.

Injections of crude MEX-organ extracts produced concentration of the red pigment in the dispersed chromatophores of eyestalkless animals. The strength and duration of the response were related to the concentration of the extracts, but the speed of the response did not change. Full contraction could be detected 15/20 min after the injection. The reaction after injections of boiled MEX extracts was stronger, and the chromatophoric responses induced by lyophilized extracts were comparable to those induced by sinus gland or total eyestalk extracts (Fig. 19).

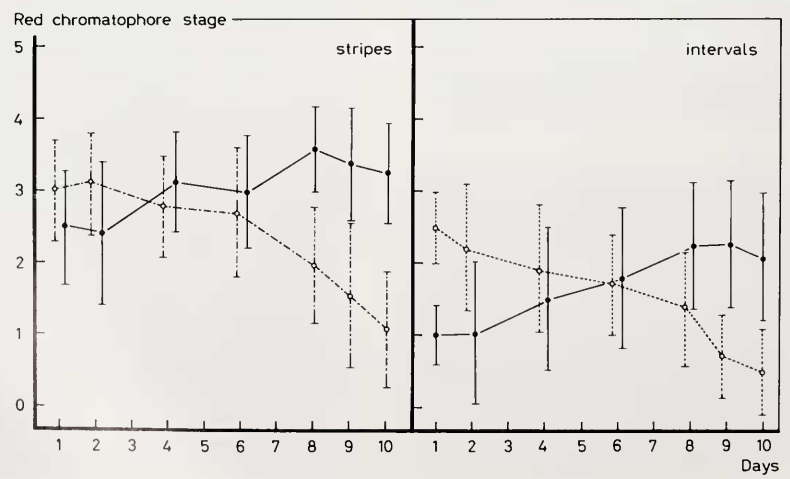


FIGURE 17. Mean dispersion, determined daily, of red chromatophores on stripes and intervals between stripes in experimental prawns kept on a white background. Solid lines: MEX extirpated prawns, dashed and dotted lines: sinus-glandless prawns. Each point represents mean of 10 animals. Chromatophore stages refer to Panouse's scale (see in text: procedure for physiological assays). Vertical bars represent ± one standard deviation (SD) from the mean.

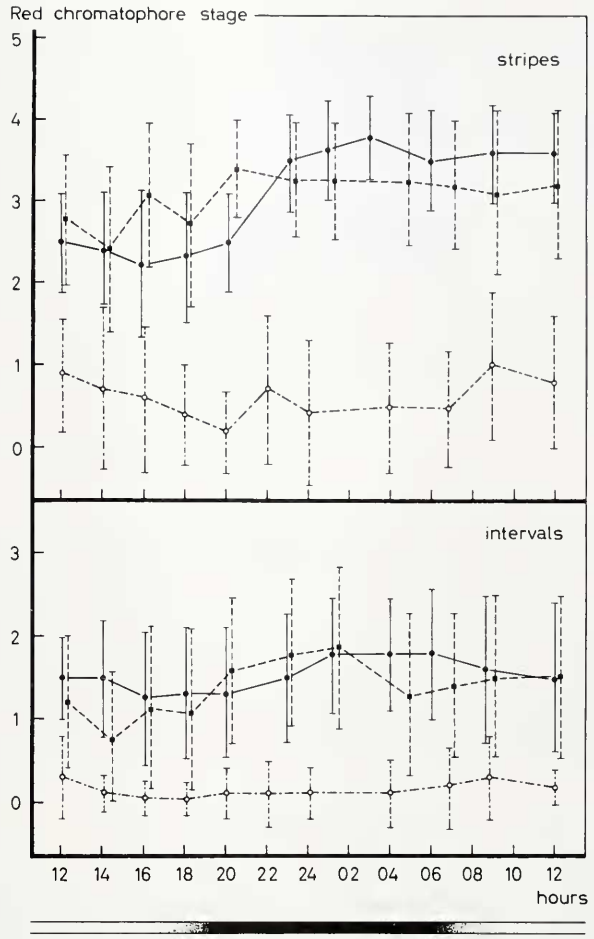


FIGURE 18. Circadian rhythm in red chromatophores (on stripes and in the intervals between stripes) of MEX extirpated and control prawns, kept on a white background. Controls were observed on the first day (dashed and dotted lines), MEX extirpated animals on the first (solid lines) and eighth (dashed lines) days. Each point represents mean of 15 animals, observed in March. Vertical bars: ±1 SD.

Glycemia determination: In a number of decapod Crustacea, blood glucose level is controlled by a hyperglycemic hormone (CHH) released by the sinus gland. In *Palaemon serratus* the neurosecretory cells that may be the source of this hormone have not been identified. To test the role of the MEX neurosecretory cell group in blood sugar regulation, lyophilized MEX or sinus gland extracts were injected into eyestalkless intermolt prawns. The glucose concentration in the hemolymph showed a 15-fold increase after sinus gland injection (232.5 ± 97.5 μg/ml versus 17.5 ± 2.0 μg/ml in control animals injected with sea water). Injection of lyophilized MEX organs caused a less dramatic rise in blood glucose (33.5 ± 18.5 μg/ml).

The influence of MEX extirpation was studied in premolt prawns, which normally have a high blood glucose level (30 to 40 g/ml). After MEX organ removal, the level was still this high 12 h after the operation, but by 2 days after MEX organ removal it had decreased sharply (17 μg/ml).

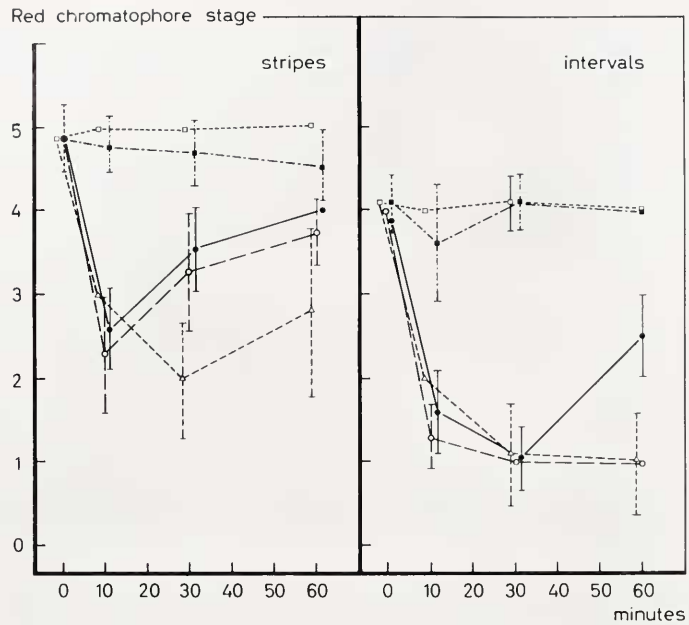


FIGURE 19. Effect of injected lyophilized extracts of eyestalks (shorter-dashed lines), MEX organs (solid lines), and sinus glands (longer-dashed lines) on dispersed red chromatophores (stripes and intervals) of eyestalkless prawns. Injections of muscle extracts (dashed and dotted lines) and sea water (dotted lines) were compared. Each point represents the mean of 10 animals; vertical bars: ±1 SD. Injection: 1 structure equivalent/animal.

DISCUSSION

General description of the MEX organ

Like the MTGX organs, the MEX organ appears distinctly at metamorphosis (Bellon-Humbert *et al.*, 1978). Most neurosecretory cells in the medulla externa form a well defined group in Natantia such as *Pandalus borealis* (Carlisle, 1959), *Palaemon serratus* (Humbert, 1965; Van Herp *et al.*, 1977), *Palaemon paucidens* (Hisano, 1974, 1976), and *Penaeus japonicus* (Nakamura, 1974).

However, in other Natantia, such as *Typhlatya garciai* (Juberthie-Jupeau, 1976) and *Atyaephyra desmaresti* (Boissou *et al.*, 1976), the neurosecretory cells are more or less scattered in the medulla externa. In other Crustacea, such as the Brachyura (Enami, 1951; Bliss and Welsh, 1952; Matsumoto, 1958; Smith and Naylor, 1972) or Anomura (Farges, 1975a; Bursey, 1975), the MEX organ is more compact. In Macrura, only scattered neurosecretory cells were described in the medulla externa (Durand, 1956; Andrew *et al.*, 1978; Shivers, 1967; Van Herp, 1972).

In Crustacea lacking a distinct MEX organ, the neurosecretory cells are mainly in two locations: above and on the opposite side of the sinus gland. In a few Natantia, neurosecretory cells are in the medulla externa near the sinus gland (Boissou *et al.*, 1976). They can aggregate to form a distinct organ, as in *Pandalus borealis* (Carlisle, 1959). In *Palaemon serratus*, the MEX organ is localized on the rostrodorsal edge of the medulla externa, at the junction with the medulla interna. Only a restricted number of neurosecretory cells are visible on its ventral side, near the distal part of the organ of Bellonci.

Light microscopy showed two to four cell types in the MEX organ. Their neurosecretory nature was established after appropriate staining, *e.g.* with P.A.F. Some small cells also were considered neurosecretory. However, some "stained neurosecretory bodies" may be lysosomes, as mentioned by Hisano (1976) and Andrew *et al.* (1978). One large and one small cell type can always be distinguished in the MEX organ (Miyawaki, 1956; Matsumoto, 1958; Lake, 1970; Hisano, 1974). Our ultrastructural results demonstrating two cell types with two different types of granules (diameters 96 and 130 nm) corroborate this view. A striking similarity exists between these two granule types and those (98 and 125 nm) of the sinus gland of the same species (Strolenberg *et al.*, 1977).

On the basis of histological observations of the MEX organ, we suspect a relationship between volume and number of active cells and the intermolt cycle, the size of the animals, and the season. In living prawns used for experiments, these variations were also noticeable in changes in organ color and size.

Histophysiological influence of MEX organ extirpation

An increase in the volume of the sinus gland and of the organ of Bellonci, as well as hypertrophy of the neurosecretory cells in the MTGX organs, became noticeable in stages A, B, and C after the molt following the operation.

In control animals, the volume of the sinus gland increased during premolt and decreased during postmolt. After MEX extirpation, the premolt aspect of the gland persisted, even after the molt ended; operated animals' glands then appeared twice as large as in controls kept in the same conditions.

Pyle (1943) found large quantities of acidophilic secretions in the sinus glands of premolt *Cambarus affinis* and *Homarus americanus*. This material decreased sharply after ecdysis. Gabe (1952) analyzed the sinus glands of 15 species of decapod Crustacea, including the prawns *Crangon vulgaris, Athanas nitescens*, and *Palaemon serratus*. In all species one type of secretion was more abundant during premolt than in post- or intermolt. Martin (1972), working on the oniscoid *Porcellio dilatatus*, noticed that secretory material began to fill up the sinus gland in stage C and reached a maximum in stage D. Farges (1975a) found the sinus gland in Diogenidae loaded with secretory substances in stage C_4 but empty in other stages. Armstrong (1973) observed also in the shrimp *Palaemon kadiakensis* variations in granule types correlated with the intermolt cycle. In *Astacus leptodactylus* Strolenberg (1979) observed a thickening of the basement membrane during premolt and assumed that this might act as a barrier to prevent the release of neurosecretory granules. Our own observations in extirpated prawns seem to support this assumption.

Lysis of granules with mean diameter 125 nm was observed in axon terminals. A similar phenomenon was reported by Martin (1972) in the sinus gland of *Porcellio dilatatus* after the extirpation of the median region of the protocerebron.

From these observations, it appears that this granule type originates in the MEX organ.

Some changes in the organ of Bellonci have been reported in relation to season (Carlisle, 1959) and molting stage (Drach and Gabe, 1963; Daguerre de Hureaux, 1967; Kauri, 1976). It is generally believed that the role of the organ of Bellonci is not exclusively sensory.

The increase in volume of the organ of Bellonci in the operated animals was related to the presence of droplets, staining orange with azan, between the perineural tissue and the degenerated onion bodies. A comparable secretion was noticed

in *Pandalus borealis* by Carlisle (1959) in the organ of Bellonci of females during vitellogenesis and in males approaching sex reversal. Drach and Gabe (1963) described in *Squilla mantis* the elaboration of acidophilic droplets from vacuolated onion bodies, reaching a peak during postmolt (B_2). This was afterwards confirmed by electron microscopic observations (Jacques, 1969; Jacques and Chaigneau, 1972). Farges (1975b) observed in Diogenidae different stages of degeneration of onion bodies and formation of large clumps ("mottes"). Using electron microscopy, Chaigneau (1976, 1977) described dense clumps of a few microns in the supporting cells, and interpreted them as remnants of degenerated onion bodies.

The production of granules by supporting cells was also suggested by several light microscopic and ultrastructural studies (Lake and Ong, 1972; Kauri and Lake, 1972; Dahl and Kauri, 1976; Chaigneau and Juchault, 1974).

Cyclic variations in the activity of the neurosecretory cells of the medulla terminalis have been linked with the intermolt cycle. Thus Drach and Gabe (1962) distinguished two maxima and two minima in the activity of the MTGX organs of *Palaemon serratus*. Our morphometric analysis is in agreement with these data.

Extirpations of the sinus gland by Passano (1951b) in several species of Brachyura and by Bliss and Welsh (1952) in *Gecarcinus lateralis* were followed by extensive storage of neurosecretory material in the vicinity of the MTGX. In some cases, the activity of these neurosecretory cells was so high that the accumulated material was referred to as a "false sinus gland" (Passano, 1951a).

Our experimental data indicate that removing one neurosecretory center, in this case the MEX organ, induces hypertrophy in other neurosecretory cell groups, such as the MTGX organs.

It is difficult to interpret the striking changes in the aspect of the main and larval sensory pores after MEX extirpation. Little is known about innervation and cyclic variations of the sensory pore X organ. Farges (1975b) described in Diogenidae a very important connection between the sensory pore and a neurosecretory cell group located at the junction of the medullae externa and interna. In the eyestalk of *Astacus leptodactylus*, there is a nervous connection between a sensory formation and the medulla interna during larval and adult life (Van Herp *et al.*, 1979). The changes observed in the sensory complex after MEX extirpation may have been induced by interruption of some connection between these structures or by other alterations of the intercellular relations.

Physiological effects

Humbert (1965) reported the influence of the pars distalis of the X organ on chromatophores. She repeated these experiments on the same species but from a different geographic population. Evidently, extirpation of the organ of Bellonci by a dorsal approach (between the medullae and the lobe of the medulla terminalis) also included the MEX organ, while a ventral approach affected a portion of the MTGX 2 organ. It now appears from our selective removal of the MEX organ that the role ascribed to the organ of Bellonci in pigment physiology should be attributed to the MEX organ, removed in the same extirpation.

The MEX organ is involved in control of red pigment. The way it acts on red pigment concentration can be compared to the role of the sinus gland. If red pigment concentrating hormone (RPCH) is carried by the 125 nm granules, then lysis of this granule type after MEX organ extirpation could explain the progressive darkening of the operated animals. But other possibilities exist. According to Shivers (1967), small granules may contain chromatophoric hormones. We found such granules (96 nm) in the MEX organ and the sinus gland. In the latter they were

apparently not affected by removal of the MEX organ. If Shivers' hypothesis is correct, the apparent thickening of the basement membrane observed in the premolt sinus gland could delay the release of granules. Perhaps these granules are partially produced by other neurosecretory cells. Our injection experiments also provide evidence for RPCH in the MEX organ. However, the concomitant role of the distal retinal pigments must be elucidated. The nighttime dispersion of distal retinal pigment after the operation could be induced by the lack of DRPH in the sinus gland. It could also result from a change in the release of this hormone. A change in the blood stream, as suggested by Kleinholz (personal communication) is also a possible cause. However, with the same conditions of extirpation and healing, neither sinusglandectomy nor sham operations generated this effect. Though the ommatidia did not seem affected on the days following extirpation, ultrastructural changes induced in the retina by a long-lasting dark adaptation are possible, as reported by Eguchi and Waterman (1979) in *Procambarus clarkii.*

Alterations in retinal pigment behavior might elicit changes in the release of chromatophorotrophins and in chromatophore behavior. This would explain rhythm reversal in operated animals. In these animals, the persistence of a rhythm in the erythrophore response may result from direct action of light on the chromatophores, in the absence of the chief hormonal control, or from the production of RPCH according to a different rhythm by other neurosecretory cell groups (MTGX organs, brain, postcommissural organs).

By applying disc electrophoresis to sinus gland extracts from *Orconectes limosus, Pacifastacus leniusculus,* and *Carcinus maenas*, Keller (1977) found that the CHH material accumulated in the sinus gland is at least 10% of total sinus gland proteins. In comparison, no CHH activity could be detected in electropherograms of medulla terminalis extracts. We were able to provoke hyperglycemia in *Palaemon serratus* by injecting crude sinus gland extracts, but not by injecting MEX material. From this we conclude that CHH is in the sinus gland, but not in the MEX organ. As demonstrated by Van Wormhoudt *et al.* (1978) in a study of the hyperglycemic activity of various endocrine structures in *Palaemon serratus*, a hyperglycemic effect can be obtained after injection of MTGX (1 and 2) material. We therefore suggest that hyperglycemic hormone is produced, not in the MEX organ, but in the MTGX organs, and that it is stored in the sinus gland. CHH-containing cells have been found in the most distal portion of the X organ in the medulla terminalis of *Astacus leptodactylus* (Van Herp and Van Buggenum, 1979), and *Carcinus maenas* (Jaros and Keller, 1979) by an immunocytochemical procedure.

In numerous decapod Crustacea, glucose levels in the hemolymph decline after epedunculation. Moreover, Hamann (1974) described a lower glucose level in *Orconectes limosus* after selective extirpation of the sinus gland. We cannot explain satisfactorily the lower glucose concentration in the blood of *Palaemon serratus* 48 h after MEX extirpation. One possibility is a nonspecific effect induced by extirpation of an arbitrary center in the eyestalk. But the nighttime migration of the distal retinal pigment may be responsible indirectly for a lower glucose level. A circadian rhythmicity in the regulation of the glucose level has been demonstrated by Hamann (1974) for *Orconectes limosus* and by Strolenberg *et al.* (1978) for *Astacus leptodactylus.*

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